PB87-116430

Influence of Ozone on Pentobarbital Pharmacokinetics in Mice

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before compl

1. REPORT NO. EPA/600/J-85/451	2.
4. TITLE AND SUBTITLE: INFLUENCE OF OZONE ON PENTOBARBITAL PHARMOCOKINETICS IN MICE (Journal Version)	5. REPORT DATE: 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): J. A. Graham, D.B. Menzel*, L. Mole, F.J. Miller, & D.E. Gardner	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: *Duke University, Durham, NC 27710 EPA/HERL/ITD/TB/MD-82, RTP, NC 27711	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: HEALTH EFFECTS RESEARCH LABORATORY - RTP, NC OFFICE OF RESEARCH AND DEVELOPMENT U.S. ENVIRONMENTAL PROTECTION AGENCY RESEARCH TRIANGLE PARK, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE: EPA/600/11
15. SUPPLEMENTARY NOTES: Toxicology Letters, 24 (1985) 163-170 #2 & #3, Feb. & March	

16. ABSTRACT

It had been shown that 3 to 5 hr exposures to ambient concentrations of ozone (O_3) increase pentobarbital-induced sleeping time in female mice, hamsters, and rats without decreasing hepatic cytochrome P-450 levels or selected mixed function oxidases. To elucidate potential mechanisms involved, clearance of pentobarbital from the blood of O_3-exposed mice was examined. Pentobarbital clearance followed first-order kinetics with a one-compartment model. Mice exposed to 1960 $\mu g/m^3$ (1 ppm) for 5 hr had a 71% increase in the plasma half-life of pentobarbital. It therefore appears possible that pentobarbital-induced sleeping time is increased due to a decreasc in hepatic metabolism of pentobarbital.

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 11
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE

EPA/600/J-85/451
JOURNAL ARTICLE

Toxicology Letters, 24 (1985) 163–170
Elsevier

TOXLett. 1356

INFLUENCE OF OZONE ON PENTOBARBITAL PHARMACOKINETICS IN MICE

(Ozone; pentobarbital; mice; pharmacokinetics)

JUDITH A. GRAHAM, DANIEL B. MENZEL*, M. LEONARD MOLE, FREDERICK J. MILLER and DONALD E. GARDNER**

*U.S. Environmental Protection Agency, Health Effects Research Laboratory, Inhalation Toxicology Division, Toxicology Branch, Research Triangle Park, NC 27711 and *Departments of Pharmacology and Medicine, Duke University, Durham, NC 27710 (U.S.A.)*

(Received September 4th, 1984)
(Accepted December 1st, 1984)

SUMMARY

Previous studies have indicated that acute exposure to ambient concentrations of ozone (O_3) as low as 196 $\mu g/m^3$ (0.1 ppm) increases pentobarbital (PEN)-induced sleeping time in female mice. To elucidate potential mechanisms involved, additional studies were performed. A 3 h exposure to 9800 μg O_3/m^3 (5 ppm) did not affect brain concentrations of PEN at time of awakening, even though sleeping time was increased. Exposure for 3 h to 9800 μg O_3/m^3 (5 ppm) did not alter the pattern of brain or plasma metabolites of PEN. Pentobarbital clearance followed first-order kinetics with a one-compartment model. Mice exposed to 9800 μg O_3/m^3 (5 ppm) for 3 h had a 106% increase in the plasma half-life of pentobarbital; at 1960 μg O_3/m^3 (1 ppm) for 3 h, a 71% increase was observed. It therefore appears possible that PEN-induced sleeping time might be increased due to an decrease in hepatic metabolism of PEN.

INTRODUCTION

5-h exposures to 1960 $\mu g/m^3$ (1 ppm) ozone (O_3) have been shown to increase PEN-induced sleeping time [1, 2] in female, but not male, mice and rats. Female hamsters exhibited a similar response and were more sensitive than males [2]. O_3

Abbreviations: HPLC, high-pressure liquid chromatography; PEN, pentobarbital.

**Current address: Environmental Sciences, Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709 (U.S.A.).

Disclaimer: This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

© Elsevier Science Publishers B.V.

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

also increased thiopental- and hexobarbital-induced sleeping time and zoxazolamine-induced paralysis time in mice [3]. There are many factors which could be responsible for the ability of O_3 to increase PEN-induced sleeping time. Since the liver is the primary site of metabolism of PEN to inactive metabolites [4–7], it is possible that the liver might be one of the target sites of O_3. Distribution also plays a role in drug action. In addition, PEN action is exerted by interaction with brain receptor sites [8], so O_3 may be affecting these receptors. Because these, as well as other factors, could be involved in the response to O_3, a series of additional investigations was performed.

Mice pretreated with mixed function oxidase inducers with different mechanisms of action exhibited roughly equivalent increases in PEN-induced sleeping time after a 5 h exposure to 1960 μg O_3/m^3 (1 ppm) [3]. Hepatic enzymes were also measured in female mice exposed acutely to concentrations of O_3 which increased PEN-induced sleeping time [9]. No significant alterations were observed in cytochrome P-450 levels or in the activities of *p*-nitroanisole *O*-demethylase or aminopyrine *N*-demethylase. There was a small increase in the activity of aniline hydroxylase which was interpreted as having no relationship to the PEN-induced sleeping time effects.

The studies to be reported here were undertaken to further elucidate the mechanism of the effect of O_3 on PEN-induced sleeping time by investigating the sensitivity of the brain to PEN and the in vivo clearance rates of PEN after O_3 exposure.

MATERIALS AND METHODS

Animals and pollutant exposure

CD-1 female mice, 5–6 weeks old, weighing between 20–25 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used. Mice were housed and exposed to O_3 as described previously [2]. On a given day, mice were exposed to air and O_3 under identical conditions. Animals were housed individually within a stainless steel Rochester-type chamber. O_3 was generated from 100% O_2 by an ozonizer. Pollutant concentrations were monitored continuously by chemiluminescence analyzers which were calibrated every 2 weeks using the photometer technique for O_3 [10]. All exposures were initiated so that they would terminate at 1:00 pm ± 15 min and all in vivo portions of PEN experiments were conducted in a temperature (24 ± 1°C) and humidity (46 ± 2% relative humidity) controlled room.

Sources of Chemicals

[^{14}C]PEN, ethyl-5-(1-methylbutyl) barbituric acid, 5-[ring-α-^{14}C], specific activity 1 μCi/4.4 μg, was obtained from New England Nuclear (Boston, MA); PEN, sodium pentobarbital (Nembutal), was obtained from Abbott Laboratories (Stone Mountain, GA). Radiochromatography of [^{14}C]PEN, performed as described in the

following, indicated the presence of a peak that eluted from the column 30 s to 1 min prior to the PEN peak. Typically, this peak was approximately 10–13% of the PEN peak. Phenobarbital and NaPEN for use as standards were obtained as crystalline solids from Sigma Chemical Co., (St. Louis, MO). Acetonitrile and methanol were ultraviolet HPLC grade from Burdick and Jackson Laboratories, Inc. (Muskegon, MI) and Matheson, Coleman, and Bell (Norwood, OH), respectively. All other chemicals were at or above reagent grade purity.

Plasma and brain concentrations of [^{14}C]pentobarbital and its metabolites at time of awakening

After exposure for 3 h to either air or 9800 μg O_3/m^3 (5 ppm), mice were injected i.m. with 24.7 mg PEN/kg. The PEN solution contained 2.25 mg Na PEN/ml and 0.22 mg [^{14}C]PEN/ml in a vehicle of 1.8% propylene glycol and 45% ethanol in physiological saline. The specific activity of the final solution was 1 μCi/49.4 μg PEN, and each mouse received 500 μCi [^{14}C]PEN/kg. At the time of awakening, blood was collected in a heparinized pasteur pipet using retro-orbital eye puncture. When bleeding was complete, brains were removed and weighed. Samples from each mouse were kept on ice and processed and measured individually.

After a 5 min centrifugation (5000 × g) of blood, plasma was collected and fortified with an internal standard of PEN and phenobarbital in methanol. The samples were precipitated with acetonitrile, centrifuged as before, and the supernatant was removed and evaporated to dryness under N_2. After resuspension in methanol, PEN and its metabolites were measured by HPLC.

Brains were fortified with an internal standard of PEN and phenobarbital in methanol, homogenized (motorized glass-glass tissue homogenizer) in ethyl acetate, filtered through Whatman No. 1 filter paper, and evaporated to dryness using a Rotavapor-R (Brinkman Instruments). Then the samples were resuspended in methanol, centrifuged as before, and the supernatant assayed for PEN and its metabolites by HPLC.

[^{14}C]Pentobarbital brain and plasma kinetics

Immediately after the 3 h exposure to air or 9800 μg O_3/m^3 (5 ppm), mice were injected i.m. with 50.2 mg PEN/kg. The PEN solution contained 4.8 mg NaPEN/ml and 0.2 mg [^{14}C]PEN/ml in a vehicle of 3.8% propylene glycol and 0.96% ethanol in physiological saline. The specific activity of the final solution was 1 μCi/100.4 μg PEN, and each mouse received 500 μCi [^{14}C]PEN/kg. Separate groups of mice, which had been exposed to air or O_3 with the above mice, were injected with 50 mg PEN/kg (no radionucleotide) for determination of PEN-induced sleeping time. At each of the selected time periods after injection (3, 5, 7, 10, 30, 60, 90 min), blood and brains were harvested from 1 mouse/sampling time, except for the 5 min time when samples were obtained from 2 mice.

Techniques for obtaining and extracting plasma were similar to those described

above. Brains were fortified with an internal standard of PEN and phenobarbital in methanol and homogenized in 0.1 M citrate buffer (pH 5). After mixing with ethyl acetate and centrifugation, the organic layer was passed through anhydrous sodium sulfate and evaporated to dryness. Samples were resuspended in methanol, centrifuged, and the supernatant assayed using HPLC. Extraction efficiency from brain and plasma was approximately 67%.

High pressure liquid chromatography measurements of [^{14}C]pentobarbital

All measurements with [^{14}C]PEN were made on an Altex HPLC (Model 332 Analytical UV detector with a Model 110A pump, Berkeley, CA), with a pre-packed reverse-phase column (Altex Ultrasil ODS, 10 μm, 4.6 mm I.D., C_{18}) monitored at 254 nm. According to the technique of Kabra et al. [11], a mobile phase of 21.5% acetonitrile in pH 4.4, 1.67×10^{-4} M K_2HPO_4 buffer was used at a flow rate of 3 ml/min. Over a 25 min period, column effluent was collected at 30 s intervals with a fraction collector (ISCO, Model 1200 pump). Preliminary studies had shown that all the ^{14}C-material passed through the column by 20 min. Aliquots of the elutate were mixed with a liquid scintillation cocktail (Beckman Ready-Solv) and counted in a liquid scintillation counter (Beckman LS 100C).

Data for each sample were normalized for extraction efficiency based upon phenobarbital peak heights for each sample compared to the internal standard run on HPLC the same day as a given mouse sample was run. Counts per min were converted to μg PEN based on the specific activity of the PEN solution injected and counting efficiency of the scintillation counter.

Plasma kinetics of non-radiolabeled pentobarbital

Mice were exposed for 5 h to either air or 1960 μg O_3/m^3 (1 ppm) and injected i.m. with 50 mg PEN/kg. At the indicated times, blood was drawn in the same manner as described earlier. Plasma was processed for HPLC measurement of parent PEN using a slightly different technique with an extraction efficiency > 98%. The chromatograph consisted of a Model M-6000 A pump, a Model U6K injector, a 30 cm × 3.9 mm μ Bondapak C_{18} reversed-phase column and guard column (Waters Assoc., Milford, MA) and a Model SF-770 variable wavelength detector (Schoeffel Inst., Westwood, NJ). The mobile phase of methanol/acetonitrile/water (147:253:600 by vol.) was used at a flow rate 1.2 ml/min, and the effluent was monitored at 195 nm Abs.

Statistical analyses

For the experiments in which the brain and plasma concentrations of PEN were determined at various time periods post injection of air- and O_3-exposed mice, the pharmacokinetics were examined using exponential models of the form $Y = \alpha e^{-\beta t}$. Here Y represents the brain or plasma concentration of pentobarbital, time equals t, and α and β are parameters which characterize the exponential model. Since the

model is a nonlinear function of the parameters, nonlinear estimation techniques were used to fit the curves [12]. Least squares estimates of the parameters were obtained using the iterative Marquardt [13] method. The data could not be resolved into a two-compartment model in large part due to the extremely good fits (>94% of the total variation) of the one-compartment models. Treatment differences between the model parameters (α and β) were determined by when the asymptotic confidence limits for a given parameter did not overlap for the two groups.

RESULTS

Effect of 9800 μg/m³ (5 ppm) on [¹⁴C]pentobarbital and its metabolites in plasma and brain

Exposure to 9800 μg O_3/m^3 (5 ppm) for 3 h caused no significant effects on the levels of PEN or its metabolites in the brain or plasma at time of awakening from sleep following a PEN injection (Table I), even though the O_3-exposed mice awakened 56.8 min later than the air-exposed animals.

In the experiment to assess the influence of a 3 h exposure to 9800 μg O_3/m^3 (5 ppm) on PEN pharmacokinetics, PEN-induced sleeping time was determined on separate groups (n = 10) of animals using nonradiolabeled PEN. The PEN-induced sleeping time was 29.3 min ± 2.3 (S.E.) in the air group and 48.0 min ± 0.4 (S.E.) in the O_3 group, a difference significant at $P = 0.0001$. Ozone caused no shift in metabolite patterns.

After i.m. injection of [^{14}C]PEN, brain concentrations of PEN gradually increased, reaching a peak at 5 min for the air group and 10 min for the O_3 group. Therefore, only the data at 10 min post-injection and later were analyzed statistically. The clearance of PEN from the brain was exponential and fit a one-compartment model in both the air ($y = 112.5e^{-0.031t}$) and O_3 ($y = 104.3e^{-0.023t}$) groups. The model explained 94.3% of the variation of the air group data and 99.8% of the variation of the O_3 group data. In view of the small sample size used in this experiment, the difference in the clearance curve was at $P < 0.1$. Half-lives of PEN were 22.4 min in the air-exposed mice and 30.1 min in the O_3-exposed animals.

TABLE I

EFFECT OF 5 PPM O_3 ON CONCENTRATIONS OF [^{14}C]PENTOBARBITAL AND METABOLITES IN BRAIN AND PLASMA AT TIME OF AWAKENING

Compound	Exposure	Brain (μg/g wet wt.)	Plasma (μg/ml)
Pentobarbital	Air	7.20 ± 1.20[a]	7.71 ± 0.82
	O_3	6.70 ± 0.95	7.92 ± 1.67
Metabolites	Air	8.76 ± 1.44	54.52 ± 3.78
	O_3	8.14 ± 0.82	59.69 ± 5.88

[a]All data are mean ± S.E. of 5 mice/group.

Peak concentrations of [^{14}C]PEN appeared in the plasma earlier than in the brain so the data were analyzed from 7 min onwards. As for the brain, disappearance of PEN from the plasma was exponential and fit a one-compartment model in both the air ($y = 104.6e^{-0.033t}$) and O_3 ($y = 81.2e^{-0.016t}$) groups of mice. The model explained 97.7% and 99.3% of the variation in the data for the air and O_3 groups. These curves were different at $P < 0.1$. The plasma PEN half-lives were 21.0 min for the air-exposed mice and 43.3 min for the O_3-exposed mice.

Effect of 1960 μg O_3/m^3 (1 ppm) on pentobarbital kinetics

Because the experiment with [^{14}C]PEN indicated a potential increase in the plasma half life of PEN, but no major change in metabolite patterns, another experiment was designed to determine quantitatively the effects of a lower concentration of O_3 on the disappearance of PEN from the plasma. In all kinetic studies, PEN clearance from the blood was first-order with one compartment. In mice exposed to 1960 μg O_3/m^3 (1 ppm) for 5 h, the half-life of PEN was increased by 71% (Fig. 1). The exponential model explained 97.8% of the variation in the air group and 97.3% of the variation in the O_3 group. O_3 exposure resulted in a decrease ($p = 0.06$) in the y intercept (the apparent volume of distribution) and in a decrease ($p = 0.07$) in the slope of the clearance curve.

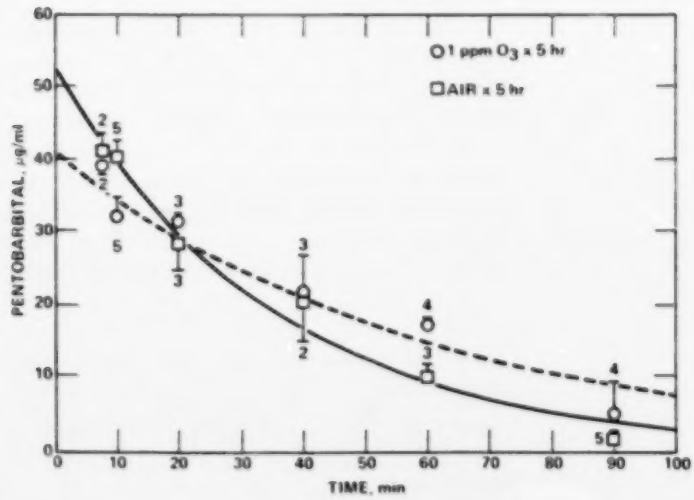

Fig. 1. Effect of 1 ppm $O_3 \times 5$ h on plasma concentrations of pentobarbital. For the air group (solid line), the exponential model was $y = 53.81e^{-0.0288t}$, and the $t_{1/2}$ was 24.0 min. For the O_3 group (dashed line), the exponential model was $y = 41.4e^{-0.0169t}$, and the $t_{1/2}$ was 41.0 min. The numbers atop the mean and standard error bars are the number of mice examined in the 2 replicate experiments.

DISCUSSION

Jori et al. [14] and Kato et al. [15] have shown that for a given dose of PEN, brain concentrations of PEN are constant at the time of awakening, irrespective of the length of sleeping time. The results of the current study showing that O_3 did not alter PEN concentrations at time of awakening, even though PEN-induced sleeping time was elevated significantly, suggests that O_3 did not alter the sensitivity of the brain to PEN.

Pentobarbital is metabolized primarily by hepatic cytochrome P-450 as opposed to cytochrome P-448 [5, 16]. Major metabolites, the diastereoisomers of PEN alcohol [5-ethyl-5-(3′-hydroxy-1′-methylbutyl) barbituric acid], were first identified in dog urine [6] and were confirmed in man [17, 18]. In the studies reported here, O_3 caused no change in the pattern of metabolites detected in the brain or plasma of mice. Unfortunately, no descriptions of the identity of mouse metabolites were found in the literature and studies in man and dogs did not use HPLC. Most likely, the major metabolite is PEN alcohol.

Examination of the PEN kinetics in plasma indicate first-order clearance which was also observed in mice by others [19–21]. In the first experiment with [^{14}C]PEN, a 3-h exposure to 9800 μg O_3/m^3 (5 ppm) delayed clearance as indicated by the 34% and 106% increase in the half lives of PEN in the brain and plasma, respectively, compared to air-exposed mice. A lower concentration of O_3 (1960 μg/m^3, 1 ppm × 5 h) increased the half life of non-radiolabeled PEN in plasma by 71%. A one-compartment model was observed in both brain and plasma over 90 min. in all experiments. With an i.m. injection, it is likely that the clearance curve observed reflects absorption, redistribution, and metabolism concurrently, with the latter being predominant at later sampling times. Thus, the differences in the plasma clearance curves and the half-lives between the air- and O_3-exposed animals are consistent with the possibility that O_3 decreased metabolism of PEN. Because of the involvement of the liver in PEN metabolism [4–7], it is suggested that the liver may be a target site of O_3 exposure. However, thiopental-induced sleeping time was also increased by 5 h exposure to 1960 μg O_3/m^3 (1 ppm) [3] and the apparent volume of distribution decreased, so it appears that drug redistribution might also be altered by O_3. Thus, the actual mechanism(s) responsible for the effect of O_3 on PEN-induced sleeping time are still unknown. Because of the regional pulmonary deposition of O_3 and the high reactivity of O_3 with lung tissue [22], it is very unlikely that the O_3 molecule itself was responsible. Reactive intermediaries of O_3 [23] are the more likely causative agents, although their participation is speculative at present.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the expert technical assistance of Margaret Carris, Margaret Grady, Jacqueline Stanislaw, Joseph W. Illing, Mary C. Daniels, and Loretta Holder.

REFERENCES

1 D.E. Gardner, J.W. Illing, F.J. Miller and D.L. Coffin, The effect of ozone on pentobarbital sleeping time in mice, Res. Commun. Chem. Pathol. Pharmacol., 9 (1974) 689–700.

2 J.A. Graham, D.B. Menzel, F.J. Miller, J.W. Illing, and D.E. Gardner, Influence of ozone on pentobarbital-induced sleeping time in mice, rats, and hamsters, Toxicol. Appl. Pharmacol., 61 (1981) 64–73.

3 Graham, J.A., D.B. Menzel, F.J. Miller, J.W. Illing and D.E. Gardner, Effect of ozone on drug-induced sleeping time in mice pretreated with microsomal inducers and inhibitors, Toxicol. Appl. Pharmacol., 62 (1982) 489–497.

4 J.R. Cooper and B.B. Brodie, Enzymatic oxidation of pentobarbital and thiopental, J. Pharmacol. Exp. Ther., 120 (1957) 75–83.

5 F.W. Ossenberg, M. Peignoux, D. Bourdiau, and J.P. Benhamou, Pentobarbital pharmacokinetics in the normal and in the hepatectomized rat, J. Pharmacol. Exp. Ther., 194 (1975) 111–116.

6 E.W. Maynert and J.M. Dawson, Ethyl (3-hydroxy-1-methylbutyl)barbituric acids as metabolites of pentobarbital, J. Biol. Chem., 195 (1952) 389–395.

7 H.D. Christensen, L. Barnett and F.I. Carroll, Biological activity of pentobarbital metabolites, J. Pharm. Sci., 62 (1973) 1722–1723.

8 S.C. Harvey, Hypnotics and sedatives, the bariburates. In The Pharmacological Basis of Therapeutics (L.S. Goodman and A. Gilman, Eds.), MacMillan, New York, 1975, pp. 102–123.

9 J.A. Graham, D.B. Menzel, R. Ward, F.J. Miller and D.E. Gardner, Influence of ozone and of nitrogen dioxide on hepatic microsomal enzymes in mice, J. Toxicol. Environ. Health., 9 (1982) 849–856.

10 U.S. Environmental Protection Agency. [6560-01-M] [FRL 1018-3] Part 50 - National primary and secondary ambient air quality standards. Calibration of ozone reference methods. Fed. Reg., 44 (1979) 8821–8833.

11 P.M. Kabra, H.Y. Koo and J. Marton, Simultaneous liquid chromatographic determination of 12 common sedatives and hypnotics in serum, Clin. Chem., 24 (1978) 657–662.

12 A.J. Barr, J.H. Goodnight, J.P. Sall and J.T. Helwig, in A User's Guide to SAS 76, Sparks Press, Raleigh, NC, 1976, pp. 193–199.

13 D.W. Marquardt. An algorithm for least-squares estimation of nonlinear parameters, J. Soc. Indust. Appl. Math., 11 (1963) 431–441.

14 A. Jori, A. Bianchetti and P.E. Prestini, Relations between barbiturate brain levels and sleeping time in various experimental conditions, Biochem. Pharmacol., 19 (1970) 2687–2694.

15 R. Kato, A. Takanaka and K. Onoda, Individual difference in the effect of drugs in relation to the tissue concentration of drugs, Jap. J. Pharmac., 19 (1969) 260–267.

16 M. Jacobson, A.Y.H. Lu, E. Sernatinger, S. West and R. Kuntzman, The effect of altering liver microsomal CO-binding hemoprotein, composition on pentobarbital-induced anesthesia, Chem.-Biol. Interact., 5 (1972) 183–189.

17 B.B. Brodie, J.J. Burns, L.C. Mark, P.A. Lief, E. Bernstein and E.M. Papper, The fate of pentobarbital in man and dog and a method for its estimation in biological material, J. Pharmacol. Exp. Ther., 109 (1953) 26–34.

18 B.K. Tang, T. Inaba and W. Kalow, N-Hydroxylation of pentobarbital in man, Drug Metab. Dispos., 5 (1977) 71–74.

19 W. Nelson and F. Halberg, An evaluation of time-dependent changes in susceptibility of mice to phenobarbital injection, Neuropharmacology 12 (1973) 509–524.

20 K.E. Becker, Jr. and I.K. Ho, Pentobarbital distribution in discrete bran areas of tolerant and non-tolerant mice, Res. Commun. Chem. Pathol. Pharmacol., 18 (1977) 23–28.

21 I. Yamamoto, I.K. Ho and H.H. Loh, The antagonistic effects of 5-ethyl-5(3-hydroxy-1-methyl-butyl)-barbituric acid on pentobarbital narcosis in both naive and tolerant mice, Life Sci., 22 (1978) 1101–1111.

22 F.J. Miller, D.B. Menzel and D.L. Coffin, Similarity between man and laboratory animals in regional pulmonary deposition of ozone, Environ. Res., 17 (1978) 84–101.

23 D.B. Menzel, Toxicity of ozone, oxygen, and relation, Annu. Rev. Pharmacol., 10 (1970) 379–394.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.